



Pharmacognostic and Anti-Stress Evaluation of Aqueous and Ethanolic Leaves Extract of *Malvastrum coromandelianum*

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ABSTRACT

The study was based on the Pharmacognostic and Anti-Stress Evaluation of Aqueous and Ethanolic Leaves Extract of *Malvastrum coromandelianum*. The fresh leaves of *M. coromandelianum* were collected from Unnao region and authenticated by the botanist at BSI, Prayagraj (Ref. no. 2024-25/549). The leaves were washed making dust-free and dried at room temperature or shade. The dried leaves were rendered into coarse powders and then finally into fine ones. The powder was weighed and soaked into ethanol and distilled water, separately for fifteen days with gradual stirrings. The obtained slurry of mixture is dried under partial vacuum using a rotary evaporator. Preliminary phytochemical screening was done. It was evaluated the anti-stress activity by following screening models i.e., Neuroprotective properties of aqueous and ethanolic extract of *M. Coromandelianum* against H₂O₂-Induced Cytotoxicity in SH-SY5Y Cells and Effect of *M. Coromandelianum* on HSP47-transformed cell viability models. The percentage yield of *M. coromandelianum* leaves extract was calculated as 43.28% and 52.54% in aqueous and ethanolic extract, respectively. In conclusion, aqueous and ethanolic leaf extract of *Malvastrum Coromandelianum* exhibited the anti-stress potential at different concentrations used in the study. In both the evaluation parameters i.e., Neuroprotective effect against H₂O₂-Induced Cytotoxicity in SH-SY5Y Cells and Cell viability/Stress effect using HSP47 Assay. Thus, it might be utilized in the management of stress; it's anti-stress role may be due to presence of flavonoids, saponins etc. This study also suggests to identify the active constituents which is responsible for the anti-stress activity.

Keywords: *Malvastrum coromandelianum*, anti-stress, HSP47 Assay, H₂O₂-Induced Cytotoxicity.

INTRODUCTION

Stress is defined as a person's physical, mental, and emotional reaction to a certain stimulus, often known as a "stressor." Stress is our bodies' way of responding to any type of demand [1]. Headaches, severe fatigue, nausea and vomiting, diarrhoea, tachycardia, chest discomfort, elevated blood pressure, flushing or disorientation, shortness of breath, restlessness, choking sensation, or hyperventilation are some of the other symptoms [2]. Eustress, distress, acute stress, and chronic stress are some of the several types of stress. Our bodies respond to stress by going into fight or flight mode [3]. The fight/flight reaction is divided into two stages: 1. Short term fight/flight responses 2. Long term fight/flight responses Stress hormones such as adrenaline and cortisol are released during this reaction.[4]

Plant profile

Malvastrum coromandelianum (False mallow), a native of tropical America, is widely dispersed in the tropics and subtropics of the world and is regarded as a highly invasive weed species of the Malvaceae family [5]. This plant species is particularly abundant on India's peninsular and disturbed Western Ghats seacoasts [6]. It is a 60 to 90 cm tall, upright, woody, annual herb or undershrub. The leaf blade has an acute apex and is ovate-lanceolate, petiolate (0.7-3cm), pilose, with lanceolate stipules (5-7mm). The solitary, axillary flower with a pilose pedicel (3 to 5mm). The calyx is accrescent and cup-shaped (5-7mm). Yellow and ovate petals. The fruit has 8-12 tricuspidmericarps and is schizocarpic with reniform fruit [7].



Fig 1. Leaves flower & twig of *M. coromandelianum*

Taxonomy

Division	:	Angiosperms
Order	:	Malvales
Family	:	Malvaceae
Genus	:	<i>Malvastrum</i>
Species	:	<i>coromandelianum</i>

Phytoconstituents are the bioactive secondary metabolites that plants (root, stem, bark, leaf, flower, fruit, seed, etc.) naturally biosynthesize and that have sufficient antioxidant power to battle diseases and, thus, show their value in medications. So far, it has been observed that malvastrone 1 (a sesquiterpene lactone) and -sitosterol (a phytosterol) both exhibit antidiarrheal characteristics. -sitosterol is also anti-inflammatory, analgesic, antilipidemic, anthelmintic, and hepatoprotective [8].

MATERIALS AND METHODS

Experimental requirements

M. coromandelianum leaves, ethanol, water bath, distilled water, rotatory evaporator and weighing machine.

Collection, authentication, and preparation of extract

The fresh leaves of *M. coromandelianum* were collected from Unnao region and authenticated by the botanist at BSI, Prayagraj (Ref. no. 2024-25/549). The leaves were washed making dust-free and dried at room temperature or shade. The dried leaves were rendered into coarse powders and then finally into fine ones. The powder was weighed and soaked into ethanol and distilled water, separately for fifteen days with gradual stirrings. The obtained slurry of mixture is dried under partial vacuum using a rotary evaporator or water-bath. The percentage yield of the extract of the *M. coromandelianum* leaves was calculated thru below mentioned formula: [9]

$$\text{percent yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100\%$$

Preliminary phytochemical screening

The aqueous and ethanolic plant extracts will be screened for different phytoconstituents to check their presence [10][11].

Alkaloids

Extracts were dissolved individually in dilute HCl and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hagers Reagent. Formation of yellow ppt indicates the presence of alkaloids.

Glycosides

Fehling's test: With distilled water dilution, Fehling's solutions A and B were heated for one minute. There were 8 drops of plant extract added to this transparent blue solution. It was then combined with 1 ml of Fehling's solution and heated for 5 minutes in a water bath. Brick red precipitation is an indication of glycoside content.

Saponins

Foam test: About 2g of the plant extract was mixed with 10ml of distilled water and shaken vigorously for a stable persistent froth. Appearance of froth indicates the presence of saponins.

Tannins

Ferric chloride test: 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% FeCl₃ was added and observed for brownish green-black or a blue-black coloration.

Lead acetate test: 2ml of plant extract was combined with 2ml of distilled water. 0.01g lead acetate was added to this combined solution and shaken well. Development of white turbidity and precipitate indicates the presence of tannins.

Flavonoids

NaOH test: A small amount of extract was treated with aqueous NaOH and HCl, and observed for the formation of yellow orange color.

H₂SO₄ test: A fraction of the extract was treated with Conc.H₂SO₄ and observed for the formation of orange color.

Terpenoids

5 ml of the aqueous plant extract were combined with 2.0 ml of chloroform, which was then added, evaporated on the water bath, and boiled with 3 ml of concentrated H₂SO₄. As terpenoids took shape, a grey colour emerged.

Steroids

2 ml of chloroform and concentrated H₂SO₄ were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Test for Reducing Sugars and Carbohydrates

Molisch's test (General test)

To 2-3ml extract of individual solvents add few drops of α-naphthol solution in alcohol, shake and add concentrate H₂SO₄ from sides of test tube. Violet ring at the junction of two liquids.

Fehling's test

It is utilised to find decreasing sugars. Make a volume of 500 mL by dissolving 34.66 grammes of copper sulphate in distilled water (solution A). 50 grammes of sodium hydroxide and 17.3 grammes of potassium sodium tartrate should be dissolved in distilled water to a volume of up to 50 millilitres (Solution B). Prior to usage, combine two solutions in an equal volume. Fehling's A and B solution in a 1 mL mixture should be boiled

for one minute. Add the test solution in an equal amount. Heat in a pot of boiling water for 5-10 minutes. An first yellow and then a brick red hue were seen.

Standardization of herbal extracts

Organoleptic characteristics

The organoleptic characteristics of the herbal extract was determined through physical examination i.e., physical appearance, color and odor.

Physicochemical evaluations

The following physicochemical parameters were performed of the herbal extracts:

Loss on drying

2-4 g of each herbal extract is weighed accurately in a previously weighed 100 ml beaker. Then it is heated in an oven at 105°C for 5 hours. After cooling the beaker in desiccator, the weight is taken. The procedure was repeated till constant weight is obtained (Chauhan S. *et al.* 2018). Loss on drying was calculated using the following formula:

$$\text{Loss of drying (\%)} = \frac{\text{weight loss}}{\text{weight of sample}} \times 100$$

Total ash

2-4g of each herbal extract is weighed accurately in a previously ignited and tarred Silica dish. The material is spread evenly and ignited in a muffle furnace at 600°C until it is white, indicating the absence of carbon. Then the dish is cooled in a desiccator and weighed. If carbon free ash cannot be obtained by this manner, the residue on the cool dish is moisten with about 2ml of water or a saturated solution of Ammonium nitrate, dried on a water-bath, and then ignited in the muffle furnace up to constant weight (Chauhan S. *et al.* 2018). The percentage of total ash is calculated using the following formula:

$$\text{Total ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Evaluation of anti-stress activity

➤ Neuroprotective Properties of aqueous and ethanolic *M. Coromandelianum* against H2O2-Induced Cytotoxicity in SH-SY5Y Cells

SH-SY5Y cells were seeded at 1×10^4 cell/well in 96 well microplates and allowed to attach for 24 h incubation. Then cells are treated with aqueous and ethanolic *M. Coromandelianum* (1/100, 1/1000, and 1/10000) for 72 h to investigate the noncytotoxic concentrations or in combination with H2O2. In H2O2 treatment panel, SH-SY5Y cells were pretreated with aqueous and ethanolic *M. Coromandelianum* at 1/2000, 1/1000, and 1/500 for 24 h, followed by treatment with 150 μM H2O2 for 24 h. Cell viability was performed using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) [12]. Briefly, 10 μL of MTT solution (5 mg/mL in PBS (-)) was added to each well of 96-well plates and incubated for 6 h at 37°C in a 95% humidified air–5% CO2 incubator. Then the formazan was formed and later dissolved in 10% sodium dodecyl sulfate (SDS). The absorbance was determined at 570 nm using blanks (wells which contain a mixture of extract and medium without cells) as a reference. Cell viability was reported as a percentage of control cells (cells treated with medium only).

➤ Effect of *M. Coromandelianum* on HSP47-transformed cell viability

HSP47-transformed cells were plated at 1×10^4 cells/well in 100 μL of culture medium and allowed to attach and grow for 48 h at 37°C (5% CO2). Then, the cells were subjected to a heat shock for 90 min at 42°C, and recovered at 37°C during 2 h. After the recovery time cells were treated with aqueous and ethanolic *M. Coromandelianum* (1/1000, 1/500, and 1/100 v/v dilutions) and incubated for 3 h at 37°C, in 5% CO2 incubator. Then the cells were washed twice with PBS (-), and HSP47 assay was performed as described in our previous study [13]. Moreover, the effect of aqueous and ethanolic *M. Coromandelianum* on HSP47-transformed cell viability was investigated using MTT assay as previously described.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of *M. coromandelianum* leaves extract was calculated as 43.28% and 52.54% in aqueous and ethanolic extract, respectively when weight of practical yield was compared with theoretical yield.

Table 1. Percentage yield calculation

<i>M. coromandelianum</i> leaves extract	Theoretical yield (g)	Practical yield (g)	% Yield
Aqueous	100	43.28	43.28
Ethanolic	100	52.54	52.54

Phytochemical screening

The aqueous and ethanolic leaves extract of *M. coromandelianum* demonstrated rich phytochemical i.e., alkaloids, glycosides, and proteins in abundance.

Table 2. Phytochemicals of *M. coromandelianum* leaves extract

Phytochemicals	<i>M. coromandelianum</i> leaves extract	
	Aqueous	Ethanolic
Saponins	+	+
Alkaloids	+	+++
Tannins	-	-
Proteins	++	+++
Glycosides	+++	+++
Amino acids	++	++
Steroids	+	++

Absent (-), Moderate (++), Abundance (+++)

Organoleptic characteristics

Table 3. Organoleptic characteristics

<i>M. coromandelianum</i> leaves extract	Physical appearance	Color	Odor
Aqueous	Amorphous	Greenish-brown	Characteristic
Ethanolic	Amorphous	Greenish-brown	Characteristic

Loss on drying

Table 4. LOD of *M. coromandelianum* leaves extract

<i>M. coromandelianum</i> leaves extract	LOD	
	Initial (g)	Final (g)

Aqueous	4.5	2.54
Ethanolic	4.5	2.82

Determination of total Ash value

Table 5. Ash value *M. coromandelianum* leaves extract

<i>M. coromandelianum</i> leaves extract	Total Ash
Aqueous	2.16
Ethanolic	2.40

Pharmacological evaluation

➤ **Neuroprotective Properties of aqueous and ethanolic *M. Coromandelianum* against H2O2-Induced Cytotoxicity in SH-SY5Y Cells**

Aqueous leaves extract of *M. Coromandelianum* at the concentrations of 1/10000, 1/1000 and 1/100 showed the cell viability (%) as 100%, 97% and 87%, respectively. However, ethanolic leaves extract of *M. Coromandelianum* at the concentrations of 1/10000, 1/1000 and 1/100 showed the cell viability (%) as 98%, 92% and 81%, respectively.

Table 6. Cell viability (%) of aqueous and ethanolic leaves extract of *M. Coromandelianum* using SH-SY5Y Cells

Treatment	Cell viability (%)
Control	98
Vehicle	96
Aqueous leaves extract of <i>M. Coromandelianum</i> (µg)	
1/10000	100
1/1000	97
1/100	87
Ethanolic leaves extract of <i>M. Coromandelianum</i> (µg)	
1/10000	98
1/1000	92
1/100	81

The neuroprotective properties of aqueous and ethanolic leaves extract of *M. Coromandelianum* against H2O2-Induced Cytotoxicity in SH-SY5Y Cells was performed. The H₂O₂ (150 µM) demonstrated the cell viability as 57%. Aqueous leaves extract of *M. Coromandelianum* + H₂O₂ (150 µM) at the concentration of 1/2000, 1/1000 and 1/500 demonstrated the cell viability as 92%, 86% and 76%, respectively. However, ethanolic leaves

extract of *M. Coromandelianum* + H₂O₂ (150 µm) at the concentration of 1/2000, 1/1000 and 1/500 showed the cell viability as 87%, 81% and 72%, respectively.

Both, aqueous and ethanolic leaf extract of *M. Coromandelianum* exhibited the anti-stress behavior in dose-dependent manner. While, ethanolic extract was found much potent which might be due to better extractions of phytochemicals in the ethanolic solvent. Ethanol acts as amphoteric solvent which dissolves multiples of phytoconstituents flavonoids, saponins etc.

Table 7. Neuroprotective Properties of aqueous and ethanolic leaves extract of *M. Coromandelianum* against H₂O₂-Induced Cytotoxicity in SH-SY5Y Cells

Treatment	Cell viability (%)
Control	98
H ₂ O ₂ (150 µm)	57
Aqueous leaves extract of <i>M. Coromandelianum</i> (µg)+ H ₂ O ₂ (150 µm)	
1/2000	92
1/1000	86
1/500	76
Ethanolic leaves extract of <i>M. Coromandelianum</i> (µg)+ H ₂ O ₂ (150 µm)	
1/2000	87
1/1000	81
1/500	72

➤ **Effect of *M. Coromandelianum* on HSP47-transformed cell viability**

Effect of aqueous and ethanolic leaves extract of *M. Coromandelianum* on Cell viability/Stress effect using HSP47 (heat shock protein) Assay was performed. In Aqueous leaves extract of *M. Coromandelianum* + H₂O₂ (150 µm), cell viability was estimated as 79%, 58% and 48% at the concentration of 1/2000, 1/1000 and 1/500, respectively. In ethanolic leaves extract of *M. Coromandelianum* + H₂O₂ (150 µm), cell viability was estimated as 76%, 54% and 37% in the concentration of 1/2000, 1/1000 and 1/500, respectively.

Table 8. Effect of aqueous and ethanolic leaves extract of *M. Coromandelianum* on Cell viability/Stress effect using HSP47 Assay

Treatment	Cell viability/Stress effect (control %)
Control	98
Aqueous leaves extract of <i>M. Coromandelianum</i> (µg)+ H ₂ O ₂ (150 µm)	
1/2000	79
1/100	58
1/500	40

Ethanollic leaves extract of <i>M. Coromandelianum</i> (µg)+ H ₂ O ₂ (150 µm)	
1/2000	76
1/1000	54
1/500	37

It is well known that chronic exposure to stress is causative factor of free radical generation and reactive oxygen species elevation in the body. Oxidative stress is well demonstrated to be a leading factor in neuronal cell death and damage. Thus, antioxidants intake might be an effective strategy to protect the human body against stress-mediated pathologies. In this respect, phytochemicals, botanicals, and plant extracts are gaining a lot of interest as complementary supplements to fight against oxidative stress. In the current study, CSEE, a polyphenol-rich plant extract, treatment showed a significant protective effect against H₂O₂-induced toxicity in SH-SY5Y cells and against heat stress-induced in HSP47-transformed cells. Moreover, CSEE significantly reversed HSP27 and HSP90 mRNA expressions in H₂O₂- treated SH-SY5Y cells. Since the first emergence of “stress”, researchers tried to find and develop several animal models to solve this problem. The tail suspension and forced swimming tests are the most common predictive tests for screening the beneficial properties of medicinal plants [14][15].

Oxidative stress-induced neuronal cell death is related to the mitochondria-related apoptosis pathway and is the most prominent neurodegenerative diseases. Additionally, the Bax/Bcl-2 ratio is crucial in apoptosis. Recent studies reported that flavonoids, such as baicalarin, kaempferol, quercetin, and naringenin, modulated the mitochondrial apoptosis pathway, activated signal pathways to suppress apoptogenic Bax, and induced antiapoptotic proteins. Park *et al.* determined that *Glycyrrhiza uralensis* Radix extract inhibited apoptosis through caspase-3, Bax, and Bcl-2 regulation in H₂O₂-induced glial C6 cells. Similarly, we confirmed that hesperetin upregulated Bcl-2 and caspase-3 and downregulates Bax in H₂O₂-treated SH-SY5Y cells. In addition, hesperetin treatment inhibited Bax/Bcl-2 ratio escalation [16].

Neurodegenerative disorder apoptosis is characterized by morphological changes such as cell shrinkage, chromatin condensation, and nucleosome degradation. DAPI staining validated that hesperetin slightly attenuated apoptotic feature induction compared with H₂O₂ treatment alone. Oxidative stress activates the MAPK pathway, incorporating ERK, JNK, and p38. ROS is related to MAPK pathway activation, especially in neurons; oxidative stress activates JNK and p38 signalling pathways. In addition, ERK is essential for enhancing neuronal cell death [17].

CONCLUSION

In conclusion, aqueous and ethanolic leaf extract of *Malvastrum Coromandelianum* exhibited the anti-stress potential at different concentrations used in the study. In both the evaluation parameters i.e., Neuroprotective effect against H₂O₂-Induced Cytotoxicity in SH-SY5Y Cells and Cell viability/Stress effect using HSP47 Assay. Thus, it might be utilized in the management of stress; it’s anti-stress role may be due to presence of flavonoids, saponins etc. This study also suggests to identify the active constituents which is responsible for the anti-stress activity.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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